CAB INTERNATIONAL
MYCOLOGICAL INSTITUTE
LIBRARY
25 FEB 1992
GRIFFITHS, M. A
1928

G-615

SMUT SUSCEPTIBILITY OF NATURALLY RESISTANT CORN WHEN ARTIFICIALLY INOCULATED

BY

MARION A. GRIFFITHS
(Contribution from Bureau of Plant Industry)

Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. 36, No. 1 : : : Washington, D. C., January 1, 1928

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

U. S. GOVERNMENT PRINTING OFFICE : 1928

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

E. W. ALLEN, CHAIRMAN
Chief, Office of Experiment Stations

C. L. SHEAR
Senior Pathologist in Charge, Mycology and Disease Survey

A. C. BAKER
Senior Entomologist in Charge, Tropical and Subtropical Plant Insect Investigations

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

R. W. THATCHER
President, Massachusetts Agricultural College

M. J. FUNCHESS
Director, Alabama Experiment Station

G. A. DEAN
Head, Department of Entomology, Kansas Agricultural Experiment Station

EDITORIAL SUPERVISION

M. C. MERRILL
Editorial Chief of Publications, United States Department of Agriculture

All correspondence regarding articles from State experiment stations should be addressed to R. W. Thatcher, Agricultural College, Amherst, Mass.

Published on the first and fifteenth of each month. This volume will consist of twelve numbers and the Contents and Index.

Subscription price: Domestic, $4.00 a year (two volumes)
Single numbers, 20 cents
Foreign, $5.00 a year (two volumes)
Single numbers, 25 cents

If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer, and they will be supplied practically at cost. Single copies of separates may be obtained free of charge from the Office of Information, United States Department of Agriculture, until the supply is exhausted.

Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

SMUT SUSCEPTIBILITY OF NATURALLY RESISTANT CORN WHEN ARTIFICIALLY INOCULATED[1]

By MARION A. GRIFFITHS[2]

Assistant Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Certain lines of corn are resistant to natural infection by smut, *Ustilago zeae*, under field conditions. This has been shown by Jones (*6*),[3] Hayes and coauthors (*4*), Garber and Quisenberry (*3*), and Immer and Christensen (*5*), who found differences in the reaction of selfed lines of corn and their crosses to corn smut. They conclude that these differences are heritable and that by selection in self-fertilized lines it is possible to obtain lines of corn either susceptible or resistant to smut.

In the winter of 1925–26 some crosses of inbred lines of corn were tested in the greenhouse for smut resistance by the writer, using methods of inoculation slightly different from those of previous investigators (*12*), who found that lines of corn which were resistant to natural infection by smut in the field were also resistant in the greenhouse. Unexpectedly high percentages of smutted plants were obtained by the writer from a cross between naturally resistant lines of corn. In fact, the percentage of smutted plants resulting from the inoculations was about the same in the cross between the naturally resistant lines as in a cross between susceptible lines. This susceptibility of the cross between naturally resistant lines of corn led to the more detailed investigation of the problem reported in this paper.

MATERIALS AND METHODS

Seed of selfed lines of the Garrick (C. I. 207),[4] Cuban (C. I. 218), and Boone County White (C. I. 240) varieties of corn and of crosses between them were used in both greenhouse and field experiments at the Arlington Experiment Farm, Rosslyn, Va. The seed was furnished by C. H. Kyle, agronomist in the Office of Cereal Crops and Diseases. Some of the lines had been selected for resistance and some for susceptibility to smut under field conditions at the Arlington farm.

Since a virulent culture of corn smut was desired, and since it was known that different cultures differ in pathogenicity (*8*, *12*), the smut for the inoculations was obtained from different localities. In addition to the collections from the Arlington Experiment Farm, there were cultures from California, Pennsylvania, Tennessee, and

[1] Received for publication Oct. 21, 1927; issued February, 1928.

[2] The writer is indebted to R. S. Talbott, who assisted in making the inoculations and in taking the smut data, and to A. M. Hurd-Karrer and B. O. Dodge, who made helpful suggestions in the preparation of the manuscript.

[3] Reference is made by number (italic) to "Literature cited," p. 89.

[4] Accession number, Office of Cereal Crops and Diseases.

Texas. The history of each smut collection, so far as known, is summarized in Table 1.

TABLE 1.—*History of collections of Ustilago zeae used in experiments*

Collection	Locality where collected	Year received	Cultured from—	Collection	Locality where collected	Year received	Cultured from—
No. 2	Rosslyn, Va	1922	Gall tissue.	No. 34	Rosslyn, Va	1925	Gall tissue.
No. 8	State College, Pa.	1923	Single spore.	No. 35	do	1925	Do.
				No. 36	do	1925	Do.
No. 28	Tennessee	1923		No. 37	do	1925	Do.
No. 29	California	1923		No. 38	do	1925	Do.
No. 32	Rosslyn, Va	1922	Do.	No. 40–43[a]	Rosslyn, Va. (greenhouse).	1926	Do.
No. 33	Dalhart, Tex	1924	Do.				

[a] These collections were from galls from smutted plants of the naturally resistant cross, Garrick F54× Cuban F79, which had been inoculated with a conidial suspension from smut collection Nos. 8, 28, 32, 33, 35, and 38.

Conidial suspensions were used for all the inoculations, and these were obtained by growing pure cultures of the various collections in carrot decoction for 6 to 10 days. These conidial suspensions were mixed just before inoculation, except in one experiment in which they were used separately.

The plants were inoculated either by injecting the conidial suspensions into the young parts with a hypodermic syringe or by pouring the suspension into the tops of the plants before they had tasseled. In some cases both methods were used on the same plant.

RESULTS

Experiments were conducted in the greenhouse in the winter of 1925–26 and in field plots in the summer of 1926 at the Arlington Experiment Farm.

IN THE GREENHOUSE

In the first experiment plants of three F_1 crosses were inoculated. One was a cross between two naturally resistant parents, the second between a resistant and a susceptible parent, and the third between two susceptible parents. The parentage of these crosses is given in Table 2. Sixty seeds of each cross had been planted about every 8 inches in rows 9 inches apart on a greenhouse bench on December 4, 1925. The plantings were made in triplicate. The air temperature was kept close to 75°–80° F., although frequent fluctuations above and below these readings occurred.

The seedlings were inoculated when 18 days old, at which time they averaged less than a foot high. The age of the plants was reckoned from the date of planting throughout the investigation. Inoculation was made with a hypodermic syringe, using a mixture of conidial suspensions from several different smut collections (Nos. 32, 33, 34, 35, and 37). A method of inoculation described by Tisdale and Johnston (*12*) was used, the needle being inserted in the plant about 2 inches above the ground and the inoculum injected until it was forced out between the folded leaves at the top of the plant. In addition a quantity of the conidial suspension was poured into the top of each plant.

From five to seven days later chlorotic areas and incipient galls had appeared. The results of this experiment are given in Table 2.

TABLE 2.—*Reaction of 18-day-old corn seedlings in the greenhouse to infection by Ustilago zeae when a suspension of conidia was injected into them about 2 inches above the ground and an additional quantity poured into the top of each*

Variety and selfed line number	Plants naturally resistant (R) or susceptible (S)	Number of plants inoculated	Plants infected			
			With small galls or lesions on 1 or 2 leaves		With severe leaf or stalk galls	
			Number	*Per cent*	*Number*	*Per cent*
Garrick F54 × Cuban F79	R × R	158	34	21.5	3	1.9
Garrick F54 × Cuban F69	R × S	166	91	54.8	5	3.0
Garrick F90 × Cuban F69	S × S	163	54	33.1	1	.6

The data show that only 3 per cent or less of either the susceptible or the resistant crosses became severely smutted. Subsequent experiments, in which some of the smut collections were used individually for inoculations, produced high percentages of smutted plants in both resistant and susceptible lines. In addition to the severely smutted plants there were some which had either small galls or lesions on one or two leaves. The percentage of plants of the cross between resistant lines which showed these lesions or small galls was 21.5, of the cross between susceptible lines 33.1, and of the cross between susceptible and resistant lines 54.8 per cent, which indicates a difference in the resistance of the hybrids to the smut.

As the plants developed it was noted that the punctures made by the hypodermic needle 2 inches above the ground showed only in the leaves. Small galls often were present on a young leaf in the region of the needle puncture and on the tip of the next younger leaf which had not been punctured. It was obvious that the needle had not penetrated the stalk but only the tightly folded leaves. A longitudinal section through these plants showed that even at the age of 33 days (two weeks after inoculation) the apical bud was still near the level of the ground instead of 2 inches above, where the inoculations had been made. It was decided, therefore, to determine the effect of injecting the smut into the region of the apical bud. Accordingly, the severely smutted plants were removed, and the remaining plants, including those with small galls on the leaves, were reinoculated in the region of the apical bud on January 7, 1926. The needle was inserted in the plant about one-half inch from the ground, instead of 2 inches, as formerly, and the mixed conidial suspension was injected downward into the very young tissue. After 10 days, galls were appearing at the base, nodes, internodes, and on the leaves of many of these plants. The severely smutted plants were removed, and notes were made on the size, number, and location of galls on each plant.

The remaining plants, which were either smut free or had but very small galls on the leaves, were inoculated again on January 30, 1926, when about 8 weeks old, at which time the apical bud was several inches from the ground. Since the plants were larger and the internodes were elongating near the base, it was more difficult to estimate the location of the apical buds. All but four plants of each of two of the crosses became smutted as a result of this reinoculation of the tissue about the apical bud, and even these few unsmutted plants became smutted after being reinoculated on February 26, 1926.

The conidial suspensions for these three inoculations were mixtures from the same smut collections (Nos. 8, 28, 32, 33, 35, and 38). The results are shown graphically in Figure 1.

The data show that over 73 per cent of the plants of both the naturally resistant and the susceptible crosses, which were resistant to inoculation 2 inches above the ground, became smutted when the inoculum was injected about two weeks later into the very young growing tissue about one-half inch above the ground. As a result of the reinoculation of the young tissue of the remaining smut-free plants, more than 97 per cent of the original number of plants became smutted. No plant of any cross remained free from smut after a third inoculation. As all of the plants of each cross became smutted, and as the smut for these three inoculations was from the same collections, it would appear that the plants of all crosses remaining smut free after the first and second inoculations were merely smut escaping.

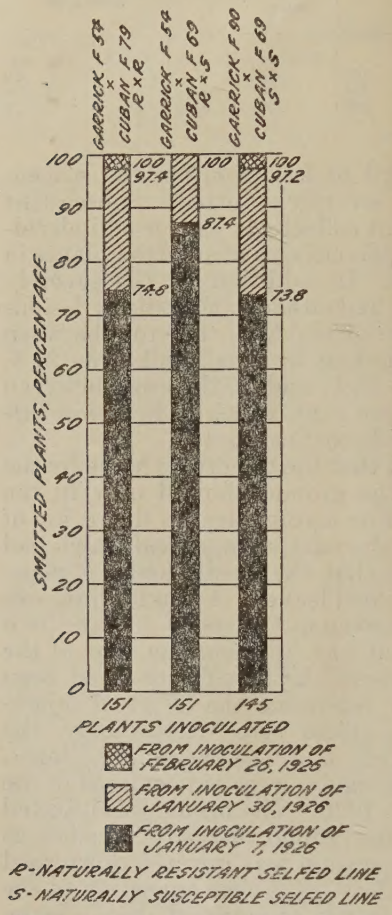

FIG. 1.—Percentages of smutted plants obtained when crosses of naturally resistant and susceptible lines of corn in the greenhouse were inoculated two or three different times by injecting conidial suspensions of *Ustilago zeae* into the region of the apical bud

Because of the unexpected susceptibility under artificial infection conditions of the naturally resistant cross, it was decided to test other lines of corn which are resistant to smut under natural conditions at the Arlington Experiment Farm. Accordingly, 40 seeds of each of five selfed lines and of a cross between two of these, all considered resistant to smut, were planted on a bench in the greenhouse on January 26, 1926. When the young seedlings were 1 month old and averaged less than a foot in height, they were inoculated about one-half inch above the ground in the young tissue about the apical bud. High percentages of plants of all the lines became smutted after this inoculation. Those plants which escaped infection were reinoculated on March 30. The two plants remaining smut free after this second inoculation became diseased after a third inoculation on April 23.

The conidial suspensions for these three inoculations were from the same smut collections as those used in the previous experiment, except that collection No. 33 was not used in the second inoculation. The data obtained from this experiment are shown in Figure 2.

After the first inoculation, 100 per cent of the plants of two of these naturally resistant lines and over 74 per cent of the plants of the other four resistant lines became smutted. After the second inoculation the percentage of smutted plants in two of these four lines rose to 100, and the percentages of smutted plants of the other two lines became 96.8 and 97.1, respectively. All of the few remain-

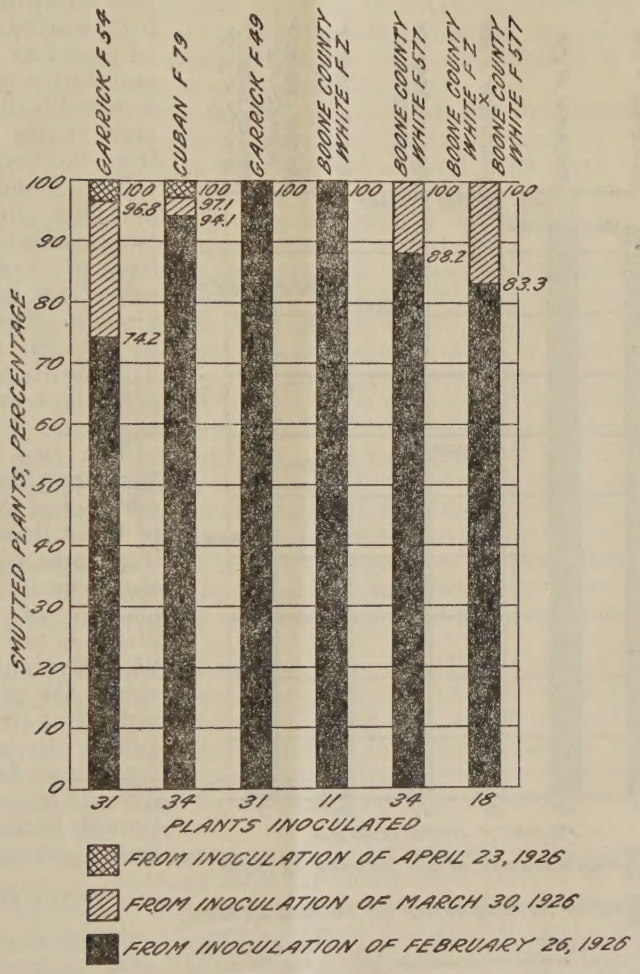

FIG. 2.—Percentages of smutted plants obtained when naturally resistant lines of corn in the greenhouse were inoculated one, two, or three different times by injecting conidial suspensions of *Ustilago zeæ* into the region of the apical bud before the period of tasseling

ing smut-free plants of these lines became smutted after the third inoculation.

In order to determine whether older plants of these same six lines of corn would become smutted after injections of conidia into the very young tissues, plants of these lines about 2½ months old were inoculated on April 23, 1926. Those plants which had not tasseled were inoculated in what was estimated to be the apical region of

the stalk. In plants which had tasseled the conidial suspension was injected through the leaf sheaths in attempts to inoculate the young bud tissues at the nodes. The percentages of smutted plants resulting from this inoculation ranged from 42.9 to 94.4. (Fig. 3.) A reinoculation of those plants which had escaped infection from the first inoculation was made on May 19, 1926. All plants with the exception of a few dwarfed or stunted ones had tasseled, and the inoculum was injected into the ears and young growths from the nodes.

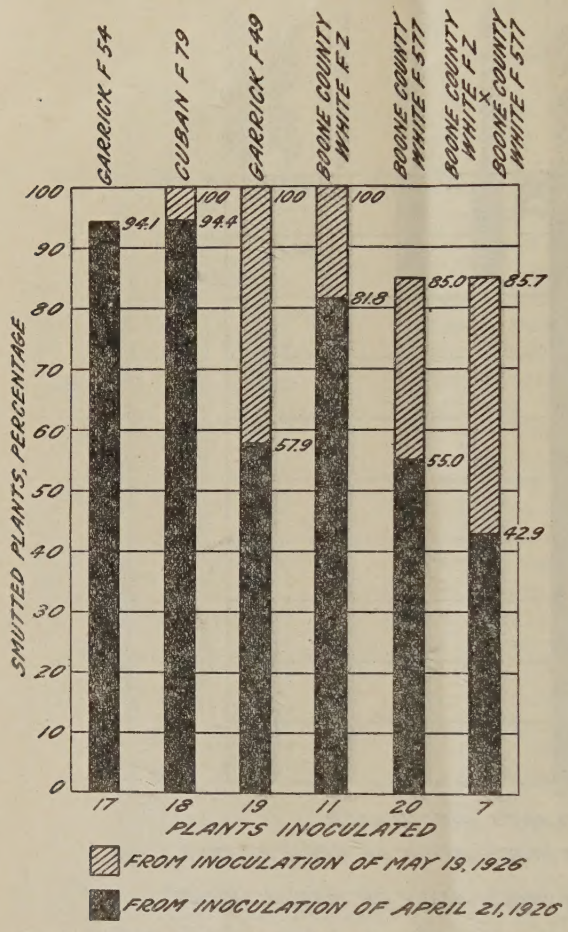

FIG. 3.—Percentages of smutted plants obtained when naturally resistant lines of corn in the greenhouse were inoculated one or two different times by injecting conidial suspensions of *Ustilago zeae* into the young tissues at about the period of tasseling

The conidial suspensions for these two inoculations were from the same smut collections used previously. (Nos. 8, 28, 32, 33, 35, and 38). The results of the inoculations are shown in Figure 3.

The two inoculations resulted in 100 per cent of the plants of three lines becoming smutted, while the lowest final percentage of diseased plants in any line was 85. Evidently the age of the plant does not affect its susceptibility to smut except in so far as the development and growth of new tissue are concerned.

IN THE FIELD

It was thought possible that the very high percentages of smutted plants resulting from the inoculation of naturally resistant lines of corn might be due to abnormal growth conditions in the greenhouse, and that in the field the plants might be resistant even if the inoculum were injected into the region of meristematic tissues. Accordingly, seeds of some of these same and other lines were planted in the field on May 20, 1926.

Injection Experiments

On July 2, when the plants were about 2 feet high, the plants of one row of each line were inoculated with a hypodermic needle as in the greenhouse experiments, using a mixture of smut collection Nos. 8, 33, 38, and 40 to 43. In order to place the inoculum in the region of the apical bud, the plants were inoculated about an inch above the ground. At the time of these inoculations smut galls had appeared on some of the plants as the result of natural infection. One row of plants of each strain was left uninoculated to serve as a check against naturally occurring infections.

Definite signs of infection as a result of the inoculations were noted in about six days. Later, the smutted plants in both the inoculated rows and the uninoculated control rows were removed. Plants with only small galls on one or two leaves were tagged but were not removed. They were counted as smut-free plants, as it was impossible to determine whether or not these slight infections resulted from the artificial inoculation. A second inoculation of the remaining smut-free plants was made on August 6, 1926, using a mixture of smut collection Nos. 29, 32, 33, 35, 36, 37, and 38. The plants at this time had not tasseled, except those of Garrick F54 × Cuban F79. Accordingly, the injections were made near the top of the plant in the youngest tissue. The location of this tissue had to be carefully estimated for each individual plant, as it naturally varied with the stage of development. As the plants of Garrick F54 × Cuban F79 had tasseled, the injections were made in the young ears and shoots only. The percentages of smutted plants are based on the total number of plants which became smutted up to and including each inoculation date. The smut percentages resulting from the two inoculations are shown in Figure 4, together with percentages of smutted plants appearing in the control rows in each of the same two periods.

The data in Figure 4 show that more than 70 per cent of the plants of every line except one were smutted as the result of the first inoculation. The one exception was Garrick F54, which had only 30.2 per cent smutted. This comparatively low percentage is interesting because the data in Figures 2 and 3 from previous experiments and the data in Tables 3 and 4 show relatively high percentages of smutted plants in this line.

The highest percentage of smutted plants in any of the uninoculated control rows of resistant lines for this period (July 2 to August 6) was 3.6, whereas in the corresponding uninoculated control rows of the susceptible lines, Garrick F90 × Cuban F69 and Cuban F69, smut percentages of 85.7 and 74.2 per cent, respectively, were obtained during the same period.

The final percentages of smutted plants resulting from the two inoculations were high in all the lines of corn tested. The percentages of smutted plants of the five naturally resistant lines totaled 100, 93.1, 91.5, 100, and 77.4 per cent, respectively. In the uninoculated control rows of these same lines the percentages of smutted plants were 5.3, 1.7, 18.0 41.8, and 4.7 per cent, respectively. That there was no lack of opportunity for natural infection was shown by the very high percentages, 100 and 98.4, of smutted plants in the two uninoculated susceptible lines. Therefore, the high percentages of

smutted plants of these naturally resistant lines seem to have resulted from the method of inoculation, the essential element of which is evidently the injection of the smut conidia directly into the very young growing tissue.

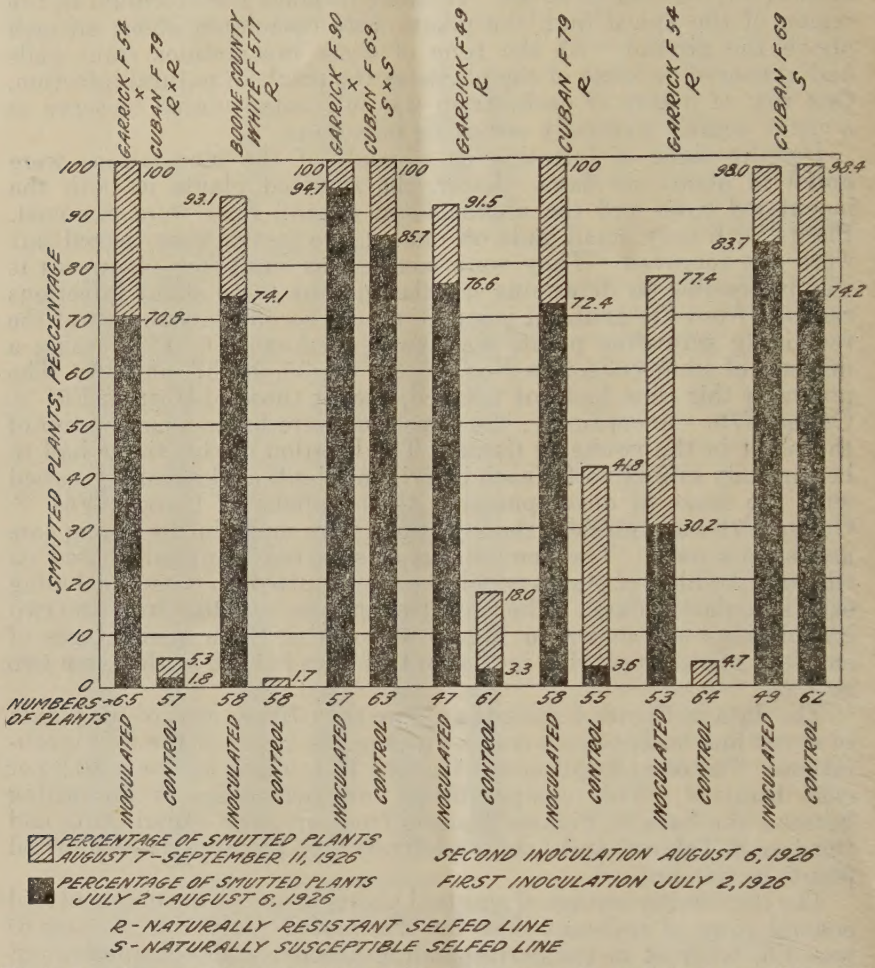
GARRICK F 54 × CUBAN F 79 R × R
BOONE COUNTY WHITE F 577 R
GARRICK F 90 × CUBAN F 69 S × S
GARRICK F 49 R
CUBAN F 79 R
GARRICK F 54 R
CUBAN F 69 S
SMUTTED PLANTS, PERCENTAGE
NUMBERS OF PLANTS
65 57 58 58 57 63 47 61 58 55 53 64 49 62
INOCULATED
CONTROL
PERCENTAGE OF SMUTTED PLANTS AUGUST 7-SEPTEMBER 11, 1926
PERCENTAGE OF SMUTTED PLANTS JULY 2-AUGUST 6, 1926
SECOND INOCULATION AUGUST 6, 1926
FIRST INOCULATION JULY 2, 1926
R-NATURALLY RESISTANT SELFED LINE
S-NATURALLY SUSCEPTIBLE SELFED LINE

FIG. 4.—Percentages of smutted plants obtained in the field from naturally resistant and susceptible lines of corn when conidial suspensions of *Ustilago zeae* were injected two different times into very young tissue; together with percentages of smutted plants in the uninoculated controls

POURING AND INJECTION EXPERIMENTS

For the purpose of comparing the percentages of smut obtainable by the method of injecting the conidia directly into the meristematic tissue with those resulting from the commonly used method of pouring the conidial suspension into the top of the plant, young plants about 6 weeks old were inoculated, both methods being used. Inoculum from the same mixed conidial suspension, consisting of collection Nos. 29, 32, 33, 35, 36, 37, and 38, was used in each case. In about half of the plants the inoculum was injected into the stalk

about 1 inch above the ground, which was estimated to be the approximate location of the young growing point in plants of this age. In the others the plants were inoculated by pouring the conidial suspension into the top. At this date a few of the plants had large galls on the crown, nodes, and leaves, and these were discarded. The results of the inoculations are given in Table 3.

TABLE 3.—*Comparative smut reaction in the field of plants of the same lines of corn when inoculated either by injecting a conidial suspension of Ustilago zeae into the apical bud tissue or by pouring the suspension into the top of the plant*

Variety and selfed line number	Plants naturally resistant (R) or susceptible (S)	Plants inoculated by—									
		Injecting inoculum into young tissue					Pouring inoculum into top of plant				
		Total	Number smutted on—			Percentage smutted	Total	Number smutted on—			Percentage smutted
			Leaf only	Stalk only	Leaf and stalk			Leaf only	Stalk only	Leaf and stalk	
Garrick F54 × Cuban F79	R × R	34	0	1	33	100	32	0	3	0	9.4
Boone County White F577	R	27	2	2	20	88.8	21	0	0	0	0
Garrick F90 × Cuban F69	S × S	31	1	2	28	100	26	0	23	1	92.3
Garrick F49	R	27	1	3	16	74.1	25	0	1	0	4.0
Cuban F79	R	26	3	6	13	84.6	24	0	1	0	4.2
Garrick F54	R	30	4	1	12	56.7	30	0	0	0	0
Cuban F69	S	34	3	6	21	88.2	28	1	13	0	50.0

The lowest percentage of smutted plants in any of the five naturally resistant lines which were inoculated by injection of the spores into the young tissue was 56.7, while in one line all of the plants were smutted. One susceptible line had 88.2 and the other had 100 per cent of plants smutted.

The highest percentage of smutted plants of any naturally resistant line inoculated by pouring the inoculum in the top was 9.4, while two lines did not show any smutted plants. The corresponding percentages of smutted plants of the susceptible lines were 50 and 92.3.

Many of the susceptible plants inoculated by pouring spores into their tops were not smutted as a direct result of the artificial inoculation but as the result of natural infection, by which many of the buds at the lower nodes later became smutted. However, it was possible to determine that the plants inoculated by injection were smutted as a direct result of the inoculation, because the smut appeared only in the parts injected and within a short time. Since the same lines of corn were used and the smut in these inoculations was from the same mixed conidial suspension, the difference in the percentages of smutted plants obviously was due to the difference in the methods of inoculation.

Injections with Local Inoculum

The purpose of this investigation was to produce smut in naturally resistant lines of corn, yet in view of reports of the existence of physiologic forms of corn smut (*8*, *11*), it seemed possible that the almost

complete susceptibility of the naturally resistant lines under artificial conditions might have resulted from the use of smut from localities other than the Arlington Experiment Farm. Accordingly, another series of inoculations was made with smut collected only at the Arlington farm, consisting of collection Nos. 2, 32, 34, 35, 37, and 38. As the plants available for these inoculations had tasseled, the inoculum was injected into the ears only. The ears were in different stages of development, some with the silk just beginning to dry and others in which the silk had not extruded from the husks. The row of each of the seven lines of corn was divided into seven sections. One section was inoculated with each of the six smut collections named above, and one was left as a control. Only plants showing smut in the ears were considered smutted as the result of the inoculations. The results, as well as the order of the six inoculations, are given in Table 4.

TABLE 4.—*Number of smutted plants in naturally resistant or susceptible lines of corn in the field when conidial suspensions of six different collections of Ustilago zeae from the Arlington Experiment Farm were injected into the ears*

Variety and selfed line number	Plants naturally resistant (R) or susceptible (S)	Uninoculated control plants		Plants inoculated with—											
				Collection No. 38		Collection No. 2		Collection No. 37		Collection No. 35		Collection No. 34		Collection No. 32	
		Total	Smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted	Number inoculated	Number smutted
Garrick F54 × Cuban F79	R × R	7	0	18	11	15	10	8	4	6	1	5	5	7	0
Boone County White F577	R	4	0	17	14	7	7	6	5	6	3	7	6	6	0
Garrick F90 × Cuban F69	S × S	2	0	3	3	3	3	1	1	2	1	2	2	4	0
Garrick F49	R	6	0	8	8	10	9	6	6	3	3	4	3	2	0
Cuban F79	R	5	0	4	4	9	9	3	3	3	1	7	6	5	0
Garrick F54	R	4	0	7	7	8	8	7	7	4	3	3	3	6	0
Cuban F69	S	2	0	3	3	1	1	1	1	1	1	3	3	5	0

The data in Table 4 show that the lowest percentages of smutted plants in any line except one were produced in the cross, Garrick F54 × Cuban F79, in which the ears were most mature at the time of inoculation. Three of the five naturally resistant lines inoculated with collection No. 2 had every plant smutted, one had 90, and the remaining one had 66.7 per cent smutted. Collection No. 2 was obtained from the Arlington Experiment Farm in 1922 and has been grown in culture since that year.

Differences in virulence of the different smut cultures were evidenced by the time of appearance of the galls, their size, and the extent of the invasion of the host tissue of the same line of corn. Collection No. 32 did not produce any smut even in the plants of the susceptible lines. The culture of smut for this inoculation grew normally in the carrot decoction. Collection No. 34 produced galls about two days earlier than the others. Collection No. 35 produced the smallest galls and the lowest percentage of smutted plants.

Although the probable importance of physiologic strains of corn smut from different localities in any corn-breeding program is recognized, it should be emphasized that the six different collections of smut from the rather localized area of the Arlington Experiment Farm showed differences in culture and in pathogenicity.

The susceptibility of these naturally resistant lines to artificial infection when inoculated with several collections of smut from the Arlington farm indicates that the high percentages of smut obtained throughout this investigation probably are not due to the fact that collections of smut from other localities were used in the inoculations, but rather to the fact that the inoculum was injected into very young tissue. Meristematic tissue of the lines tested is susceptible to smut even in plants of lines which are highly resistant to natural infection at the Arlington Experiment Farm.

DISCUSSION

In these experiments there apparently was little, if any, resistance of the very young host tissue to the smut. However, it is possible, of course, that there are physiological differences in the older tissues of the different lines of corn, which may determine their relative degree of resistance. Yet, in view of the fact that even in susceptible lines the galls are produced largely in the younger growing tissues, it is conceivable that resistance or susceptibility is largely a matter of relative accessibility of the susceptible parts to the invading organisms.

As has been shown, lines of corn developed at the Arlington Experiment Farm which were there resistant to natural smut infection in the field were extremely susceptible, under artificial inoculation, to several individual smut collections from the Arlington farm, as well as to mixed collections from different localities. These lines of corn as grown in the field at the Arlington farm in 1926 were resistant to natural infection by the smut occurring naturally there. (Fig. 4.) As stated previously, the naturally resistant lines used in these investigations were developed from only three varieties of corn. Consequently it is possible that the genetic factors involved in the selection of the various lines for resistance and susceptibility were limited.

If physiological differences in young tissue of different lines of corn are not determining factors in smut resistance, it is obvious that the injection method used in these experiments should not be employed for practical tests of the susceptibility of lines of corn to smut infection.

Recently Christensen and Stakman (*1*) have obtained differences in pathogenicity of physiologic forms of corn smut from different localities. Some of these forms were less virulent and some more virulent than the form from St. Paul, Minn., with which they were compared. These differences in pathogenicity perhaps would explain the susceptibility of the naturally resistant lines of corn developed at the Arlington Experiment Farm when inoculated with smuts from different localities, including the Arlington Experiment Farm. Yet even the existence of these forms of different pathogenicity from different localities does not explain the high percentages of smutted plants obtained in naturally resistant lines when several individual

smut collections from the Arlington Experiment Farm were used for the inoculations. (Table 4.)

It is possible that collections of smut from different localities would represent different physiologic forms. Yet even the different collections from the Arlington farm differed in infective power. Preliminary data concerning the pathogenicity of single-chlamydospore cultures of corn smut from the Arlington farm indicate that they differ even when obtained from the same smut gall.

It seems possible that some of the physiologic strains of *Ustilago zeae* may be due to crossing within the species. Sartoris (*9*) reported the conjugation of the conidia of *U. zeae* under certain growth conditions. It is not known whether these forms resulting from conjugation are pathogenic.

The significance of conjugation and hybridization in relation to physiologic forms of various species of fungi is apparent. Recent researches on smuts and other fungi indicate the advisability of using single chlamydospores, or even single conidia, of corn smut as the basis for determining physiologic forms of the organism. Kniep (*7*) has described and illustrated the conjugation between the germ tubes of sporidia of different species of Ustilago and the growth following these unions. Dickinson (*2*) recorded the occurrence of hyphal fusion within and across the two species of smut, *U. levis* and *U. hordei*. The pathogenicity of these new forms has not been demonstrated. Shear and Dodge (*10*) found that crossing the different species of the *Monilia sitophila* group by mating their haplonts resulted in the formation of what appeared to be hybrid perithecia with mature ascospores.

SUMMARY

Plants of selfed lines of corn and crosses between them which are resistant to natural infection by smut in the field at the Arlington Experiment Farm, Rosslyn, Va., were very susceptible when artificially inoculated in very young tissue. The apical and nodal bud tissues, immature ears, young leaves, and tassels were all highly susceptible when injected with conidial suspensions.

These lines were susceptible to several individual smut collections from the Arlington Experiment Farm as well as to mixed collections from there and from other localities. This fact indicates that the susceptibility of the naturally resistant lines need not be attributed to the use of physiologic forms from other localities.

LITERATURE CITED

(1) Christensen, J. J., and Stakman, E. C.
1926. physiologic specialization and mutation in ustilago zeae. Phytopathology 16: 979–999, illus.

(2) Dickinson, S.
1927. experiments on the physiology and genetics of the smut fungi.—hyphal fusion. Roy. Soc. [London] Proc. (B) 101 (B 708): 126-136, illus.

(3) Garber, R. J., and Quisenberry, K. S.
1925. breeding corn for resistance to smut (ustilago zeae). Jour. Amer. Soc. Agron. 17: 132–140.

(4) Hayes, H. K., Stakman, E. C., Griffee, F., and Christensen, J. J.
1924. reactions of selfed lines of maize to ustilago zeae. Phytopathology 14: 268–280.

(5) Immer, F. R., and Christensen, J. J.
1925. the reaction of selfed lines and crosses of maize to ustilago zeae. Phytopathology 15: 699–707.
(6) Jones, D. F.
1918. segregation of susceptibility to parasitism in maize. Amer. Jour. Bot. 5: 295–300.
(7) Kniep, H.
1926. ueber artkreuzungen bei brandpilzen. Ztschr. Pilzkunde (n. F., Bd. 5) 10: 217–247, illus.
(8) Melchers, L. E.
1921. ecologic and physiologic notes on corn smut, ustilago zeae. (Abstract) Phytopathology 11: 32.
(9) Sartoris, G. B.
1924. studies in the life history and physiology of certain smuts. Amer. Jour. Bot. 11: 617–647, illus.
(10) Shear, C. L., and Dodge, B. O.
1927. life histories and heterothallism of the red bread-mold fungi of the monilia sitophila group. Jour. Agr. Research 34: 1019–1042, illus.
(11) Stakman, E. C., and Christensen, J. J.
1926. physiologic specialization of ustilago zeae and puccinia sorghi, and their relation to corn improvement. (Abstract) Phytopathology 16: 84.
(12) Tisdale, W. H., and Johnston, C. O.
1926. a study of smut resistance in corn seedlings grown in the greenhouse. Jour. Agr. Research 32: 649–668, illus.